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Conversion of the Sex Pheromone of the Cabbage Looper

THE mechanisms by which airborne molecules stimulate the olfactory receptors of insects remain largely undefined. In the Insecta, antennal sensilla contain pores which connect the external environment with the receptor membrane of the dendritic nerve endings[1–3]. The ultimate fate of the stimulant molecule that enters the pore is not yet known. Unpublished data cited by Schneider[1] and Kaissling[4] indicated that bombykol (E)-10, (Z)-12-hexadecadien-1-ol, was progressively metabolized into acid and ester after absorption on the antennae, or other body parts of males and females of *Bombyx mori* (L.). This finding cannot be directly correlated with any specific step in the olfactory mechanism, but it suggested that an enzymatic process might be involved at some point. Other indications of chemical stimulants interacting with protein substances in the antennae of insects have been reported[5,6].

We report a protein binding process and an enzymatic process in antennal homogenates of the cabbage looper, *Trichoplusia ni* (Hübner), which may be involved in the perception of pheromone. The cabbage looper was chosen for several cogent reasons: (*a*) the behavioural responses to the sex attractant ((Z)-7-dodecen-1-ol acetate) and various related chemicals have been well studied[7,8]; (*b*) electrophysiological studies have been completed with all the chemicals used here (to be reported); and (*c*) all chemicals and pheromones were available in high purity (95% + purity; the synthetic pheromone sample contained no measurable (Z)-7-dodecen-1-ol by GLC analysis).

Antennae from male moths (400 pairs, approximately 25 mg wet weight) were homogenized in 4.0 ml. of 0.5 M sucrose buffered with 0.05 M Tris-HCl, *p*H 7.5 at 4° C. The homogenate was centrifuged at 20,000*g* for 45 min and the supernatant was decanted. The protein content was determined by the method of Lowry *et al.*[9].

Binding of the sex attractant and its analogues to soluble antennal protein(s) was measured by ultraviolet difference spectroscopy[6,10,11]. Difference spectra recorded from 220 to

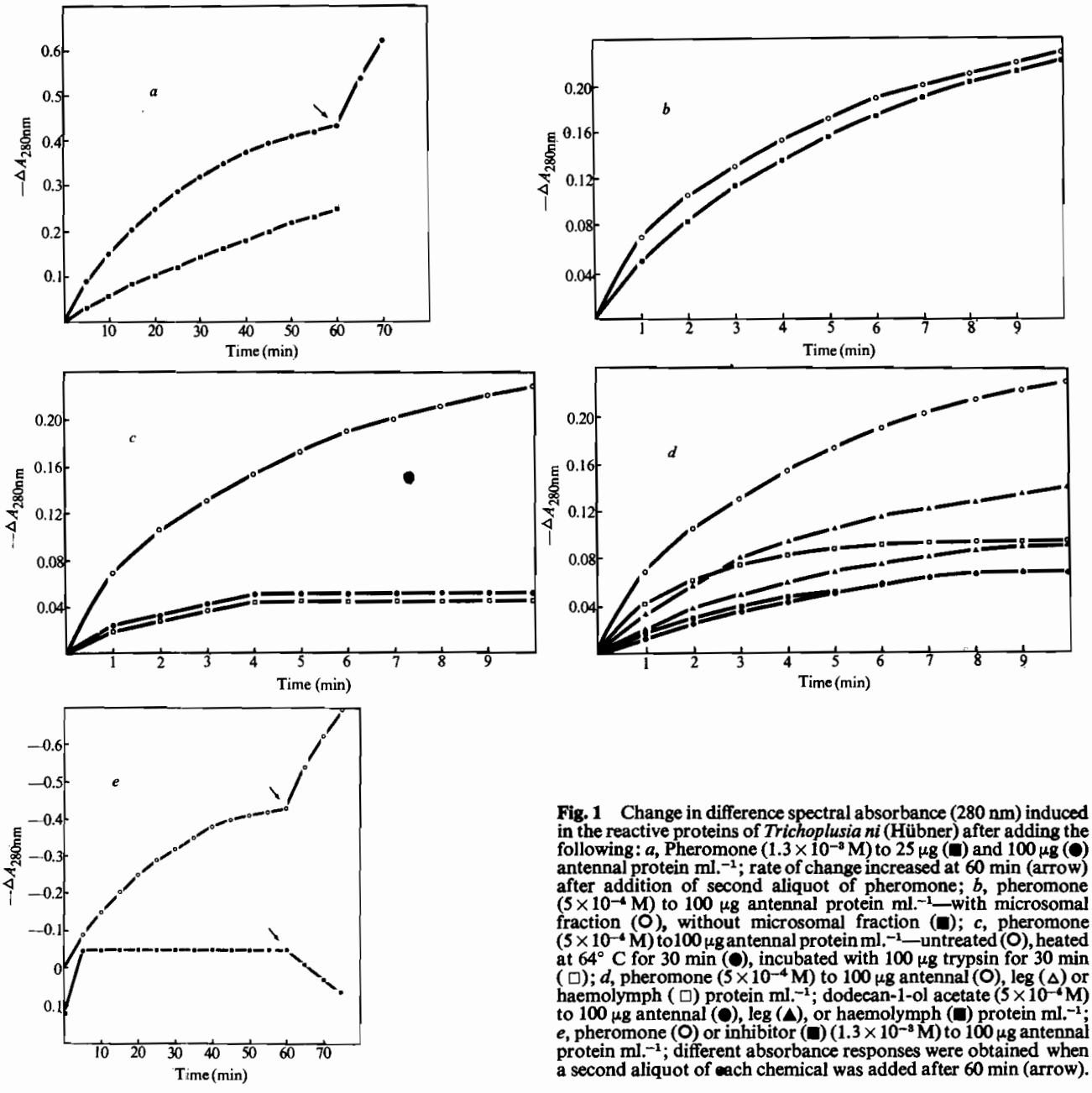

Fig. 1 Change in difference spectral absorbance (280 nm) induced in the reactive proteins of *Trichoplusia ni* (Hübner) after adding the following: *a*, Pheromone (1.3×10^{-8} M) to 25 μg (■) and 100 μg (●) antennal protein ml.$^{-1}$; rate of change increased at 60 min (arrow) after addition of second aliquot of pheromone; *b*, pheromone (5×10^{-4} M) to 100 μg antennal protein ml.$^{-1}$—with microsomal fraction (○), without microsomal fraction (■); *c*, pheromone (5×10^{-4} M) to 100 μg antennal protein ml.$^{-1}$—untreated (○), heated at 64° C for 30 min (●), incubated with 100 μg trypsin for 30 min (□); *d*, pheromone (5×10^{-4} M) to 100 μg antennal (○), leg (△) or haemolymph (□) protein ml.$^{-1}$; dodecan-1-ol acetate (5×10^{-4} M) to 100 μg antennal (●), leg (▲), or haemolymph (■) protein ml.$^{-1}$; *e*, pheromone (○) or inhibitor (■) (1.3×10^{-8} M) to 100 μg antennal protein ml.$^{-1}$; different absorbance responses were obtained when a second aliquot of each chemical was added after 60 min (arrow).

350 nm on a dual-beam recording ultraviolet-visible spectrophotometer, obtained with the antennal supernatant, the pheromone, and the pheromone analogues, indicated a time-dependent maximal negative peak at 280 nm. Subsequent measurements of the negative absorbance difference ($-\Delta A$ or negative change in optical density units) relative to the baseline at 280 nm were recorded with a single-beam ultraviolet-visible spectrophotometer. Baselines (zero absorbance (optical density) as a function of 280 nm) were attained by electronically neutralizing the original absorbance of the antennal preparation and the chemical stimulants with the absorbance control system (total absorbance negated was less than 1.0 OD unit). Full scale sensitivity of the recording system was set at 0–0.5 A, and the temperature of the cell compartment was thermostated at 20° C.

Twenty-five μl. of a sonicated solution of the pheromone in distilled water (final concentration 1.3×10^{-3} M) was added to 25 μg of antennal protein in 1 ml. of 0.05 M Tris-HCl, *p*H 7.5. The rate of absorbance change ($-\Delta A$) at 280 nm was dependent on the concentration of the antennal protein (Fig. 1*a*). As the concentration of protein was increased, the rate of change in absorbance also increased. After 1 h, the rate of absorbance change had diminished, but was reactivated by addition of another 25 μl. of pheromone.

A 2 ml. aliquot of the antennal supernatant was centrifuged at 105,000*g* for 2 h to remove the microsomes. The resulting particle-free supernatant reacted with the pheromone (5×10^{-4} M) with only a slight loss in activity (Fig. 1*b*). The activity of the antennal supernatant, however, was destroyed by heat at 64° C or incubation with 100 μg of trypsin (*p*H 7.5) at 20° C for 20 min (Fig. 1*c*). These results demonstrated that the monitored absorbance change involved interaction of the pheromone with soluble protein in the antennae. This suggests the interaction was enzymatic because the gradual change in absorbance was more characteristic of an enzymatic reaction than of nonenzymatic binding[10]. If the absorbance change had been due only to pheromone binding or complexing with antennal protein, then the change in absorbance should have rapidly reached a plateau that would have been dependent on the concentration of the two reactants, antennal protein and pheromone.

To determine whether the reaction was unique to the antennae, particle-free supernatant fractions of haemolymph and legs were prepared and assayed for pheronome reactivity (Fig. 1*d*). The leg proteins were less active than the antennal proteins, and the haemolymph proteins were even less active. No measurable reaction occurred when pheromone was incubated with bovine serum albumin. However, preparations from the antennae of females were as reactive as the male preparations. A behaviourally inactive saturated analogue of the pheromone, dodecan-1-ol acetate, was assayed with the antennal, leg and haemolymph protein preparations. There was less reaction with dodecan-1-ol acetate in all three preparations than with the pheromone. These results indicate that the reactive protein showed some specificity for the pheromone and was primarily localized in the antennae.

To demonstrate more conclusively that interaction of the pheromone with the antennal supernatant was enzymatic, we monitored the rate of pheromone disappearance during the incubation with antennal proteins by gas chromatography[12]. Sonicated pheromone in distilled water (final concentration 2.02×10^{-2} M) was incubated with 2.0 ml. of antennal homogenate (100 mg wet weight ml.$^{-1}$), 0.2 ml. aliquots were collected during the reaction at 20° C and each aliquot was extracted with 0.5 ml. of anhydrous ether. One 0.2 ml. sample was held in a boiling water bath for 15 min before a 60 min incubation and then extracted. Gas chromatography of this sample gave one peak with a retention time identical to that of the pheromone. All other samples gave two peaks, one that represented the pheromone and a second one that had a retention time identical to that of (Z)-7-dodecen-1-ol, the alcohol moiety of the pheromone, which is a potent inhibitor of male attraction to the pheromone[12]. A time-dependent reduction in pheromone peak and a simultaneous increase in the height of the alcohol peak were observed. The ratios of the pheromone peak height to alcohol peak height values were 93.0, 47.5, 15.5, 8.7, 4.0, 2.2, 1.77, after 1, 5, 9, 15, 30, 45, and 60 min, respectively. These results demonstrated an enzymatic hydrolysis of the pheromone to the alcohol. In a subsequent experiment, the relative percentage of pheromone converted by the antennae, haemolymph, and legs was 33.9, 10.1 and 6.5% per 60 min.

When the inhibitor, (Z)-7-dodecen-1-ol (final concentration $2,5 \times 10^{-3}$ M), was incubated with antennal supernatant (100 μg protein ml.$^{-1}$), it produced a rapid initial positive increase in absorbance which gradually declined to a negative value during the first 5 min, after which a plateau was reached that was stable for over 1 h (Fig. 1*e*). Addition of more inhibitor caused another, but slower, positive increase in absorbance change. This response, by contrast to that obtained with the pheromone (Fig. 1*e*), suggested that the absorbance change was due to reversible conformational transitions in proteins binding the alcohol as noted by M. Laskowski[13]. Incubation of the antennal preparation (100 μg protein) with the inhibitor (1.3×10^{-3} M) for 15 min before addition of the pheromone (1.3×10^{-3} M) did not prevent the absorbance change induced by the pheromone.

The significance of the *in vitro* conversion of the pheromone to inhibitor in the mechanism of olfaction by this insect remains obscure. There is no direct indication that this reaction is related to transduction[1]. It is possible, however, that enzymatic hydrolysis of the pheromone to the alcohol is a mechanism which regulates adaptation in the neurone and/or is a means of biologically inactivating the pheromone to prepare the dendritic receptor membrane for subsequent stimulation. This reaction may also be useful in obtaining inhibitors in other species whose mating behaviour is mediated through pheromones.

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S. M. Ferkovich
M. S. Mayer
R. R. Rutter

Insect Attractants, Behavior and Basic Biology Research Laboratory, PO Box 14565
USDA Agricultural Research Service, Gainesville, Florida 32601

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